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DIFFERENTIAL EFFECT OF NUTRIENT SOLUTIONS ON THE SIZE OF VARIOUS PARTS OF MAIZE SEEDLINGS GROWN IN THE DARK [1]

By J. H. KEMPTON

Senior botanist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

The American species of grasses belonging to the maize tribe (Tripsaceae) and at least one oriental member of the tribe, *Coix lacryma-jobi* L., have greatly elongated mesocotyls (epicotyls) when their seedlings are grown in the dark under suitable conditions of moisture and temperature.

With the object of utilizing the very open scale afforded by the mesocotyl as a measure of the growth substance and hence possibly the growth potentialities of the plant, a long series of experiments has been conducted to determine the effect of temperature and of various radiations of known wave length and energy upon the elongation of the mesocotyl.

Difficulties were encountered in producing uniform effects with given treatments, and it was soon found that for comparative results there must be a rigid control of all environmental conditions. Slight variation in temperature, above or below the optimum, produced measurable effects and, in the case of wave-length studies, slight variations in energy resulted in detectable responses.

In these studies the seedlings were grown in sterilized sand which, with washing and sterilizing, was used repeatedly. Lack of agreement between repeated experiments and excessive variability of subgroups within experiments compelled increasing refinements in control of conditions of growth and led eventually to a study of the effect of the solution upon developing seedlings.

In considering the effect of culture solutions, it should be kept clearly in mind that, although both cell expansion and division are involved, there is no growth in the sense of an increase in dry matter over that supplied by the material stored in the seed. The seedlings were in total darkness from the time of planting until they were finally measured, and therefore there was no photosynthesis. The experiments reported here were concerned only with those processes that ordinarily take place before the plumule reaches the soil surface.

ELONGATION OF MESOCOTYL

EFFECT OF TAP WATER AND COMPLETE CULTURE SOLUTION AT VARIABLE TEMPERATURE

The first test of the effect of the culture solution was a comparison of the length of mesocotyl obtained in tap water with that produced by an equal quantity of Eaton's (*3*) [2] solution modified by increasing the monopotassium phosphate (KH_2PO_4) from 3 to 30 gm. for 100

[1] Received for publication March 27, 1942.

[2] Italic numbers in parentheses refer to Literature Cited, p. —.

liters. This experiment involved 40 cans, each containing 520 gm. of oven-dry sand moistened with 110 cc. of solution or tap water. Each can was planted with 15 seeds of Funk Yellow Dent Corn (*Zea mays* L.). The entire set was arranged in a space of 2 by 3 feet in 5 blocks, each containing 4 replications of each solution. The replications within blocks were paired, randomized as to position within and between blocks. The entire set was grown in an air-conditioned room where the maximum temperature had an upper limit at 80° F. but the minimum temperature was permitted to drop to 70°. The seeds were planted September 28, and the plants were measured October 5, 1939. Only the lengths of mesocotyls were recorded. The mean length for plants grown in tap water was 99.61 ± 3.35 mm. and for those grown in the culture solution 124.75 ± 3.41 mm. The variance, apportioned on the basis of individual plant measurements, is shown in table 1.

TABLE 1.—*Analysis of variance based on length of mesocotyl of individual plants*

Source of variation	Degrees of freedom	Mean square	Source of variation	Degrees of freedom	Mean square
Total	575	3,584.59	Within subgroups	15	4,810.19
2-can groups	19	[1] 7,330.82	Solution	1	[1] 90,969.45
8-can subgroups	4	[1] 16,783.20	Error	555	3,298.87

[1] $P<0.01$.

From this analysis it is evident that the culture solutions were associated with differences in length of mesocotyl.

EFFECT OF TAP WATER AND FIVE SALT SOLUTIONS AT VARIABLE TEMPERATURE

The second test of the effect of culture solutions involved six solutions, one being tap water and the others as follows:

Complete solution:[1]	*Grams per 100 liters*
$Ca(NO_3)_2+4H_2O$	94
KNO_3	30
$(NH_4)_2SO_4$	27
$MgSO_4+7H_2O$	49
KH_2PO_4	30
No potassium:	
$Ca(NO_3)_2+4H_2O$	163.8
$(NH_4)_2SO_4$	26.4
$MgSO_4+7H_2O$	49.0
$Na_2HPO_4+12H_2O$	68.4
No magnesium:	
$Ca(NO_3)_2+4H_2O$	93.6
$(NH_4)_2SO_4$	26.4
KNO_3	30.3
KH_2PO_4	27.4
$CaSO_4+2H_2O$	34.4
No calcium:	
$(NH_4)_2SO_4$	26.4
KNO_3	70.7
KH_2PO_4	27.4
$MgSO_4+7H_2O$	48.8
No ammonium:	
$Ca(NO_3)_2+4H_2O$	117.0
KNO_3	50.5
$MgSO_4+7H_2O$	48.8
KH_2PO_4	13.7

[1] Eaton's solution modified.

In all these solutions the minor salts, as given by Eaton, were added. The experiment was set up with 96 cans, each containing 520 gm. of sterilized oven-dry sand moistened with 120 gm. of solution and planted with 15 seeds of Funk Yellow Dent corn. The cans were arranged in 16 blocks, each block containing 1 can of each solution. The arrangement of the solutions within the block was random. The experiment was located in the air-conditioned room used in the first test and the entire block of cans was covered with a tarpaulin to minimize wanton air currents that might produce variable temperature. The cans were planted December 1, and measurements of mesocotyl length were made on December 11, 1939.

The apportioned variance on the basis of individual plants is given in table 2.

TABLE 2.—*Analysis of variance based on length of mesocotyl of individual plants*

Source of variation	Degrees of freedom	Mean square
Total	1,374	1,582.90
Replicates	15	1,108.45
Solutions	5	[1] 10,920.92
Error	1,354	1,555.29

[1] $P<0.01$.

Obviously the culture solutions were associated with differences in mesocotyl length. The mean length of mesocotyl for each of the six cultures is given in the following tabulation.

Solution:	*Mean length of mesocotyls (mm.)* [1]	Solution—Continued.	*Mean length of mesocotyls (mm.)* [1]
Tap water	154.77	No magnesium	167.64
Complete solution	164.44	No calcium	156.11
No potassium	161.95	No ammonium	172.92

[1] Differences between means less than 7.56 mm. are not significant.

The mesocotyls produced in tap water are significantly shorter than those from any culture except that lacking calcium. The complete solution and the solutions lacking potassium or magnesium produced mesocotyls of comparable length, but omitting ammonium sulfate resulted in a definite increase in length of mesocotyl. The mesocotyls in this test were much longer than those of the first test, but this second experiment ran 3 days longer.

EFFECT OF TAP WATER AND SEVEN SALT SOLUTIONS AT VARIABLE TEMPERATURE

The third experiment repeated the second, with the addition of two solutions, one lacking both calcium and ammonium, the other containing only calcium salts. The composition of these new solutions was as follows:

No calcium or ammonium:	*Grams per 100 liters*	All calcium:	*Grams per 100 liters*
$NaNO_3$	45.5	$CaSO_4+2H_2O$	103.8
KNO_3	50.5	$Ca(NO_3)_2+4H_2O$	163.8
KH_2PO_4	13.7		
$MgSO_4+7H_2O$	48.8		

The experiment was set up in 96 cans, 12 for each of the 8 culture solutions. Each can contained 600 gm. of oven-dry sand moistened with 120 cc. of solution and planted with 20 seeds of Funk Yellow Dent corn. The cans were arranged in 12 groups, each group containing 1 member of each solution. The cans within groups were randomized, as were the positions of the groups. Seeds were planted December 28, 1939, and the experiment was terminated January 8, 1940. The location was identical with that of the previous experiment.

The apportioned variance on the basis of individual plants is given in table 3.

TABLE 3.—*Analysis of variance based on length of mesocotyl of individual plants*

Source of variation	Degrees of freedom	Mean square
Total	1,848	1,747.82
Replicates	11	1,351.33
Solution	7	[1] 4,930.95
Error	1,830	1,738.03

[1] $P<0.01$.

The effect produced by the solutions, although not as pronounced as in the previous test, is clearly significant. The mean length for each of the eight cultures is given in the following tabulation:

Solution:	*Mean length of mesocotyl (mm)*[1]	Solution—Continued.	*Mean length of mesocotyl (mm.)*[1]
Tap water	170. 16	No calcium	168. 45
Complete solution	177. 72	No ammonium	175. 92
No potassium	172. 19	No calcium or ammonium	165. 87
No magnesium	175. 72	All calcium	178. 59

[1] Differences between means less than 7.78 mm. are not significant.

It is evident that the maximum elongation of the mesocotyl is dependent upon calcium and is not greatly affected by any one of the other salts in the solutions used. The differences in length of mesocotyl between this test and the preceding one can be charged only to the temperature variation, as the growing period in both cases was 11 days.

ELONGATION AND DRY WEIGHT OF SEEDLING PARTS

EFFECT OF DISTILLED WATER, TAP WATER, AND THREE SALT SOLUTIONS AT TWO CONSTANT TEMPERATURES

The evident interaction of temperature and solution made it desirable to test the effect of the culture solutions under different temperatures and with more rigid temperature controls.

Two chambers were available, operating at uniform temperatures, one at 72°±1° F., the other at 85°±2°. These two temperatures are about equidistant from the optimum of 78° found for mesocotyl elongation.

Five cultures were used, namely, distilled water, tap water, Eaton's solution as given on page 184, this same solution at doubled concentration, and the calcium solution given on page 185. The seeds were planted in cans holding 600 gm. of oven-dry sand moistened with 120 cc. of solution. Ten seeds were planted in each can through a perforated disk resting on the surface. This latter was used to permit excising the seedlings at the surface free from sand so that dry weights could be obtained. The total lengths of mesocotyls, therefore, as given in this test are shorter by 2 cm. more or less than their actual lengths because of the section left in the sand.

Ten cans of each culture were planted at each temperature and randomized within temperatures. They were not disturbed from the time of planting until the plants were measured. The series at 72° F. was grown for 12 days and that at 85° for 10 days.

Three measurements of length were made on each seedling, namely, length of mesocotyl, coleoptile, and leaves. The sum of the lengths of mesocotyl and leaves gives the total seedling height. Oven-dry weights of the total product of each can were obtained separately for the mesocotyls and for the leaves, including the coleoptiles.

In order to eliminate unequal frequencies, only the means of cans were used in analyzing the data, as germination was not perfect

The variance was apportioned for each of the three measurements of length and for three measurements of weight, namely, weight of mesocotyl, weight of leaves and coleoptile, and weight per meter of mesocotyl.

The means for the several seedling parts in the different cultures at the two temperatures are given in table 4.

TABLE 4.—*Mean lengths and weights of seedlings and parts of seedlings grown in the dark in various media and at different temperatures*

Culture	Mean length, at temperature shown (° F.), of—								Mean oven-dry weight, at temperature shown (° F.), of—							
	Mesocotyl		Coleoptile		Leaves		Seedling		Mesocotyl		Leaves		Weight per meter of mesocotyl		Seedling	
	72°	85°	72°	85°	72°	85°	72°	85°	72°	85°	72°	85°	72°	85°	72°	85°
	Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Distilled water	114.9	145.2	55.4	56.6	144.7	135.4	259.6	280.6	0.0171	0.0153	0.0306	0.0265	0.149	0.105	0.0477	0.0418
Tap water	120.2	140.5	55.2	56.4	161.9	147.6	282.2	288.1	.0186	.0158	.0359	.0269	.154	.113	.0545	.0427
Eaton's	121.3	139.5	61.9	61.0	191.2	182.7	312.4	322.2	.0156	.0130	.0427	.0346	.129	.093	.0583	.0476
Eaton's × 2	134.0	152.7	72.3	68.1	201.9	183.2	335.9	335.8	.0155	.0150	.0506	.0409	.116	.099	.0660	.0559
Calcium	129.9	145.6	59.0	58.6	154.1	141.9	284.0	287.5	.0154	.0139	.0335	.0256	.118	.095	.0491	.0396
Difference required for significance (P=0.01)	13.48		4.84		25.4		23.6		.0030		.0052		.0150		.004	

The measurements and figures 1 to 8 show that the temperatures were only partly equalized by the time differential. The analyses of variance (table 5) show significant temperature effects for all measures other than length of leaves and coleoptiles. The only significant interaction of culture with temperature is that for the weight per meter of mesocotyl. The mesocotyls grown in salt solution at 72° F. are relatively lighter in weight per unit length than those grown in the same solutions at 85°.

TABLE 5.—*Analyses of variance based on means of 120 cans of 10 seedlings for the seedling parts shown*

Source of variation	Degrees of freedom	Mean square					
		Length			Weight		
		Mesocotyl	Coleoptile	Leaves	Mesocotyl	Leaves	Per meter of mesocotyl length
		Mm.	*Mm.*	*Mm.*	*Gm.*	*Gm.*	*Gm.*
Total	119	323.84	53.39	1,309.31	0.00001	0.00009	0.00060
Temperature	1	[1] 11,197.29	14.62	1,894.09	[1] .00007	[1] .00151	[1] .02600
Culture	5	[2] 611.37	[1] 608.68	[1] 12,760.55	[1] .00003	[1] .00109	[1] .00300
Interaction	5	220.07	20.55	566.76	.00001	.00003	[1] .00100
Error	108	214.65	29.56	808.13	.00001	.00003	.00028

[1] $P<0.01$.
[2] $P<0.05$.

Interactions of temperature and culture could be expected in this experiment only if the 2 days' difference in growth period allowed between the two temperature groups failed to equalize the temperature differential. The only possibility for a temperature effect independent of time would be in case of threshold effects, and these could hardly be expected with the temperature range used.

The entire experiment was somewhat disappointing in efficiency, since differences between pairs of means of less than 10 percent could not be established for most measurements. In the case of the weight of mesocotyl per unit length, differences of less than 20 percent between means of treatments are of questionable validity.

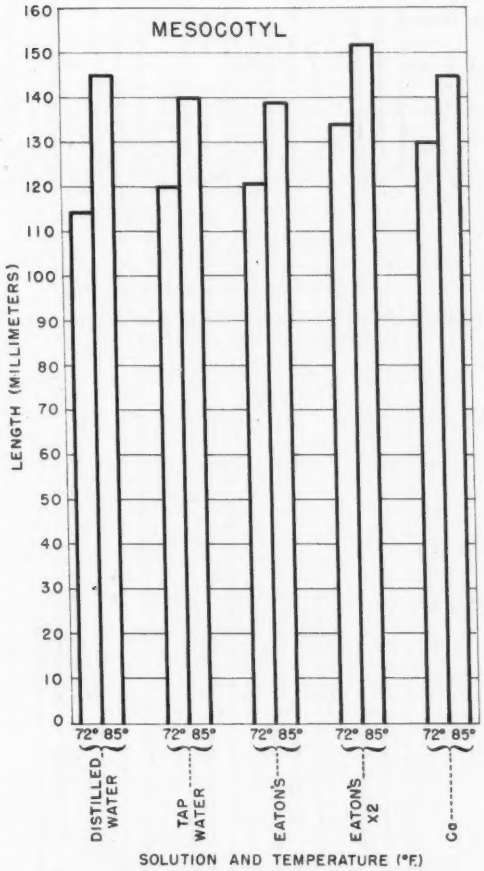

FIGURE 1.—Mean length of mesocotyls of maize seedlings grown in five cultures (p. 186) in the dark at 72° and 85° F.

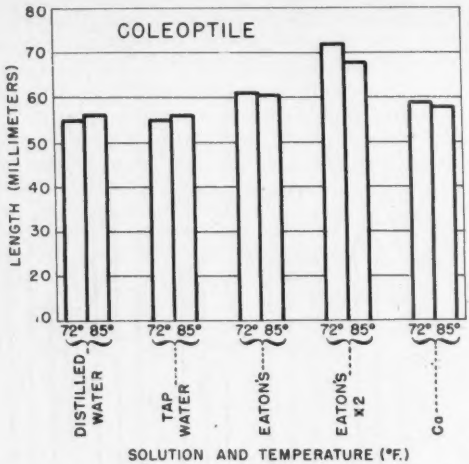

FIGURE 2.—Mean length of coleoptiles of maize seedlings grown in five cultures (p. 186) in the dark at 72° and 85° F.

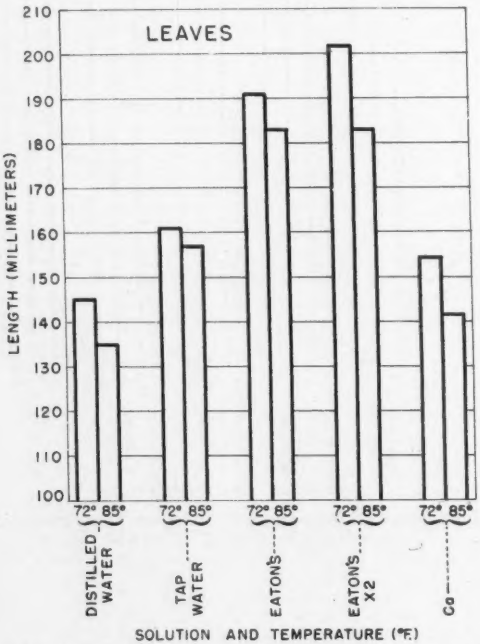

FIGURE 3.—Mean length of leaves of maize seedlings grown in five cultures (p. 186) in the dark at 72° and 85° F.

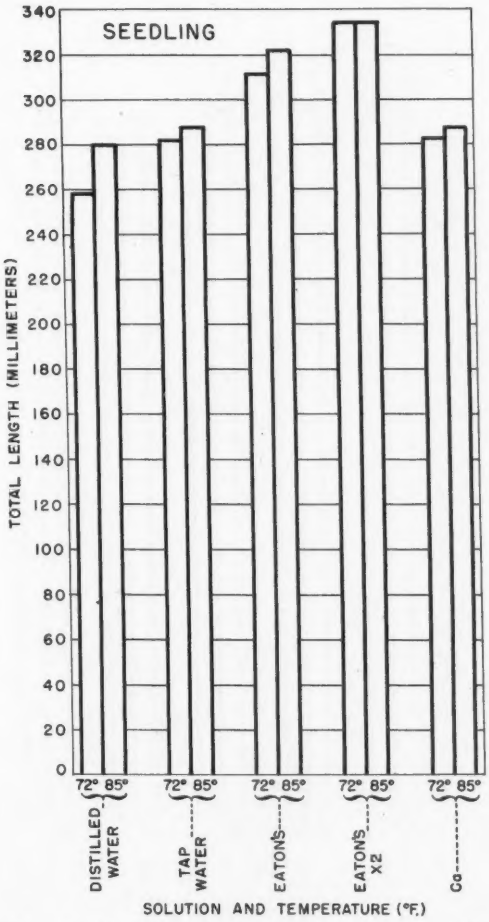

FIGURE 4.—Mean length of entire seedlings of maize grown in five cultures (p. 186) in the dark at 72° and 85° F.

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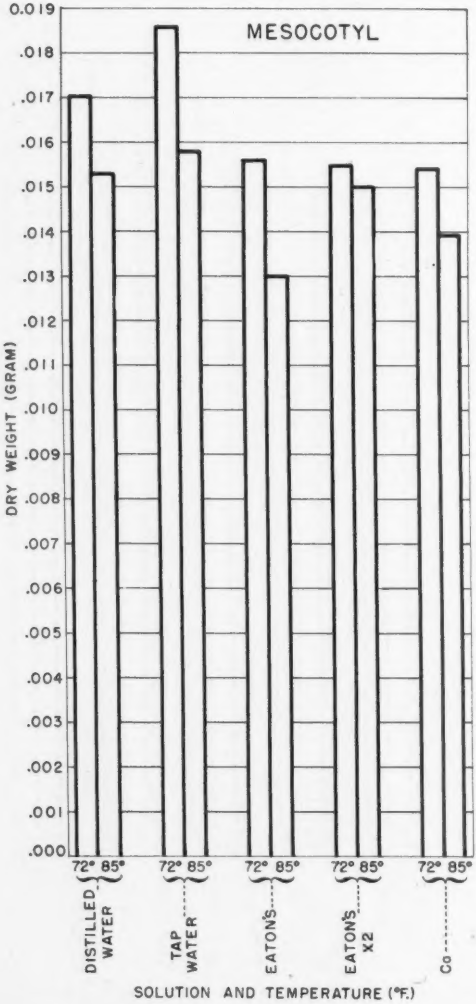

FIGURE 5.—Mean oven-dry weight of mesocotyls of maize seedlings grown in five cultures (p. 186) in the dark at 72° and 85° F.

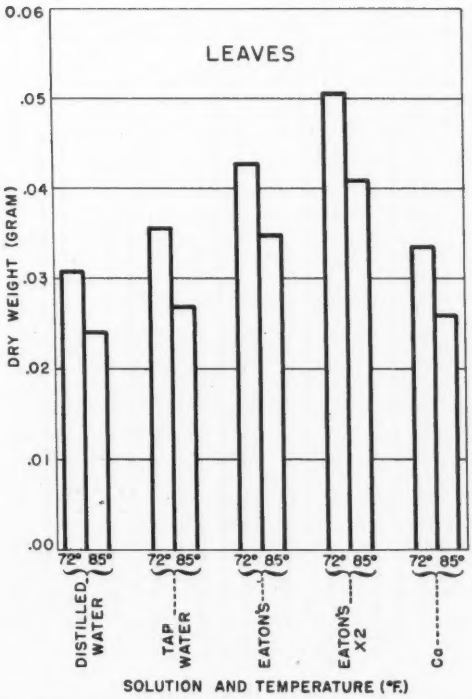

FIGURE 6.—Mean oven-dry weight of leaves of maize seedlings grown in five cultures (p. 186) in the dark at 72° and 85° F.

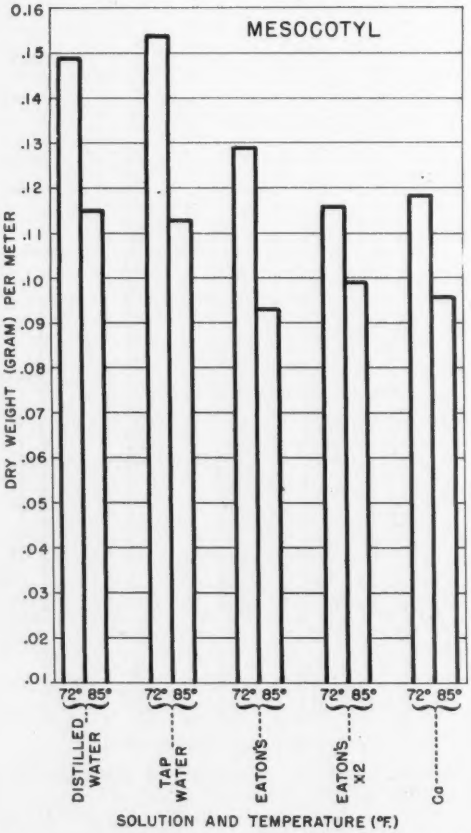

FIGURE 7.—Mean oven-dry weight per meter of mesocotyls of maize seedlings grown in five cultures (p. 186) in the dark at 72° and 85° F.

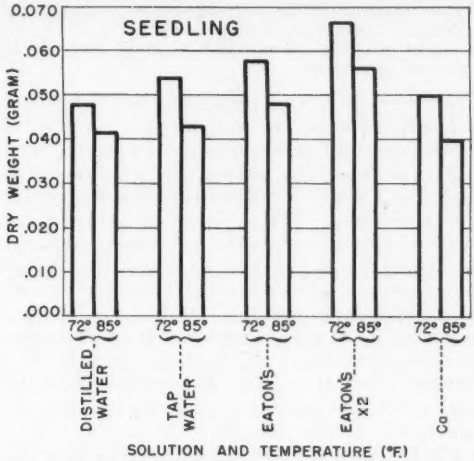

FIGURE 8.—Mean oven-dry weight of entire seedlings of maize grown in five cultures (p. 186) in the dark at 72° and 85° F.

TEMPERATURE EFFECTS

With all the cultures the length of the mesocotyl at the higher temperature was significantly greater than the length at the lower temperature for the corresponding culture. There was no temperature effect on length of coleoptile or tops or on total length.

There was a temperature effect on weight of mesocotyl such that the mesocotyls at the lower temperature were the heaviest, and this was true also for the weight of tops, which was significantly greater at the lower temperature in all the cultures except the distilled water.

In all cultures the weight per meter of mesocotyl was greater at the lowest temperature, and in every case the entire plants weighed more at the lowest temperature.

The entire plant at this stage weighed approximately 15 percent of the dry weight of the seed when planted.

SOLUTION EFFECTS

Taking the plants in distilled water as the base, the following facts are demonstrable:

(1) At the lower temperature the cultures in the concentrated solution and in the calcium solution have significantly longer mesocotyls.
(2) At the higher temperature there are no significant differences in mesocotyl length.
(3) At both temperatures the coleoptiles are significantly longer in the two complete solutions.
(4) At both temperatures the tops are significantly longer in the two complete solutions.
(5) At both temperatures the entire seedlings are significantly taller in the two complete solutions.
(6) At neither temperature are the weights of mesocotyls affected by the solutions.
(7) At both temperatures the weights of tops are significantly greater in the two complete solutions.
(8) At the lower temperature the weight per meter of mesocotyl is significantly less in the two complete solutions and in the calcium solution.

(9) At the lower temperature the seedlings grown in tap water and in the two complete solutions are significantly heavier.

(10) At the higher temperature only the seedlings grown in the concentrated solution are significantly heavier.

From the above results, it is apparent that the two complete solutions have a profound effect on the elongation of the maize seedling growing without light and on the transfer of stored material from the seed. This effect is greater at temperatures below the optimum. On the other hand, it is equally apparent that except for the size of mesocotyl the active element in the complete solution is not calcium. Calcium, however, appears to be the activating element in mesocotyl lengths, as indicated by the total length and the reduced weight per meter. This suggests that the role of calcium, insofar as it affects this organ, is one of stimulation of elongation rather than the speeding up of the translocation of stored material from the endosperm.

It may be concluded that, although calcium in the solution will insure the complete elongation of the mesocotyl, the other salts present in the complete solutions are needed for the greatest development of the plant parts above the mesocotyl.

From the reactions at the two temperatures it may be concluded that the function of the salts in the solution is to speed the transfer of stored material to the leaves.

EFFECT OF DISTILLED WATER AND COMPLETE CULTURE SOLUTION AT CONSTANT TEMPERATURE

Although it has been shown by the three previous experiments that seedlings grown in culture solutions in the dark are larger than those grown in distilled water, the possibility remains that the solutions do not increase the ultimate size of the seedlings but act merely as accelerating agents, speeding the transformation of the stored starch and the translocation of the resulting sugars from the endosperm to the seedling. It was not practicable to prolong the experiments until all seedling growth had ceased, because of the break-down of tissue from decay; therefore, in the experiments thus far conducted, if the solutions increased the rate of translocation, this would be reflected as an increase in the size of the seedling.

As a preliminary investigation of the effect of solution on the speed of seedling growth, the following experiment was started June 14 in a chamber operating at a constant temperature of 72° F. In this experiment 24 soil cans were used, each containing 575 gm. of coarse, oven-dry sand, the particles of which passed a ½-inch sieve and were caught on a ⅛-inch mesh.

Each can was planted with 20 seeds of Funk 1939 stock, but the weights of this seed were not obtained. After planting, 12 cans were wet with 110 cc. of Eaton's solution at twice the published concentration, and 12 were wet with 110 cc. of distilled water. The cans were then covered with tin tubes and placed in the 72° F. chamber. Beginning on June 19, 5 days after planting, 2 cans from each solution were removed at random daily with the exception of June 23 (Sunday), giving 6 samplings. Measurements were recorded for mesocotyls, coleoptiles, and tops. These parts, as products of single cans, together with the roots, were oven-dried separately and weighed. The data are given in table 6, and shown graphically in figures 9 to 16. The analyses of variance are given in table 7.

TABLE 6.—*Mean lengths and weights of seedling parts grown in the dark at a constant temperature of 72° F. in the media indicated*

Culture	Days from planting	Length			Oven-dry weight						
		Mesocotyl	Coleoptile	Leaves	Mesocotyl	Leaves	Roots	Seed residue	Total recovered dry matter	Weight of mesocotyl per meter	Dry matter translocated
		Millimeters	*Millimeters*	*Millimeters*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
Distilled H_2O	5	58.65	15.47	[1] 15.47	0.0128	0.0060	0.0137	0.2337	0.2662	0.2176	0.0325
Solution		63.72	18.10	18.10	.0136	.0072	.0142	.2477	.2828	.2139	.0350
Distilled H_2O	6	99.30	22.06	27.06	.0219	.0054	.0183	.2236	.2745	.2212	.0456
Solution		108.05	30.00	30.00	.0211	.0072	.0169	.2102	.2606	.2009	.0452
Distilled H_2O	7	122.42	41.94	43.50	.0256	.0161	.0231	.1973	.2622	.2095	.0648
Solution		138.95	60.02	72.01	.0257	.0266	.0259	.1742	.2525	.1855	.0782
Distilled H_2O	8	151.77	51.05	59.35	.0285	.0192	.0263	.1807	.2549	.1882	.0740
Solution		157.55	72.32	117.29	.0254	.0367	.0260	.1553	.2435	.1614	.0881
Distilled H_2O	10	171.97	58.72	82.02	.0291	.0235	.0273	.1528	.2327	.1688	.0799
Solution		172.71	75.19	172.46	.0246	.0528	.0226	.1286	.2287	.1431	.1000
Distilled H_2O	11	172.65	54.43	82.18	.0259	.0213	.0236	.1529	.2238	.1503	.0708
Solution		169.16	72.54	196.83	.0222	.0614	.0249	.1035	.2121	.1313	.1085
Difference required for significance (P=0.01)		22.70	9.90	29.70	.0052	.0450	.0030	.0300	.0273	.0300	

[1] For the first 2 samples, tops and coleoptiles are identical as no leaves were exserted.

TABLE 7.—*Analyses of variance based on means of 24 cans of 20 seedlings for the seedling parts shown*

Source of variation	Degrees of freedom	Mean square								
		Length			Weight					
		Mesocotyl	Coleoptile	Leaves	Mesocotyl	Leaves	Roots	Seed residue	Total recovered dry matter	Weight per meter of mesocotyl
Total	23	1,698.27	440.09	3,404.33	0.000027	0.00438	0.000023	0.00190	0.000491	0.000981
Culture	1	185.76	[1] 1,053.37	[1] 14,710.91	[1].000030	[1].00168	.000003	[1].00246	.000201	[1].002380
Time	5	[1] 7,578.32	[1] 1,718.90	[1] 10,321.79	[1].000110	[2].00091	[1].000096	[1].00757	[1].001891	[1].003719
Interaction	5	47.22	[1] 68.42	[1] 2,159.60	.000002	.00024	[1].000006	[2].00042	.000129	.000072
Error	12	62.23	11.02	98.47	.000003	.00022	.000001	.00010	.000083	.000102

[1] $P<0.01$.
[2] $P<0.05$.

The effect of the culture solution was greatest on the tops. At the termination of the experiment, the dry matter in the tops was at its highest point, and this is true also for the length of tops, while the plants in distilled water had attained their limit.

There was no demonstrable effect of the culture solution on weight of roots.

In conformity with the effect of solutions on seedling parts, the seed residues show that the culture solution accelerated the translocation of material from the seed and removed a greater total amount.

In weight per meter of mesocotyl the culture solution produced plants with less dry matter for unit of length in this organ. The ratio of dry matter to unit length declined with length, and therefore with time. Apparently increase in length is at least partly at the expense of diameter.

The measurements show that the experiment continued until the mesocotyls had begun to shrink in length and lose in weight. It is apparent that the culture solution used accelerated the elongation of the mesocotyl but did not increase its final length, and the numbers were too small to establish the significance of this interaction. On the other hand, the solution not only hastened the elongation of coleoptiles and tops, but it also resulted in an increase in their final length. Evidently the seedlings in the distilled water had reached their final size before the conclusion of the experiment, which would indicate that the salt solution facilitated the translocation of the stored products from the seed. It is possible that in distilled water the loss of solutes from the seed to the solution becomes a limiting factor on further translocation. However, other experiments show that the loss of dry matter to the solution is only about 5 percent more in the distilled water than in the solution.

At every sampling date after the first, the dry matter in the mesocotyls of seedlings grown in distilled water exceeded that of seedlings grown in the solution. By the third sampling date, the mesocotyls of seedlings grown in the solution had reached their maximum dry-matter content and declined from then on, probably because of the drain made by the rapidly growing tops.

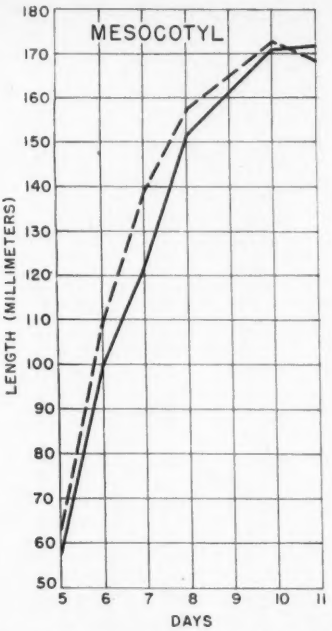

FIGURE 9.—Increase in length of mesocotyls of maize seedlings grown in the dark in two cultures at a constant temperature of 72° F. Solid line, distilled water; broken line, Eaton's solution at double concentration.

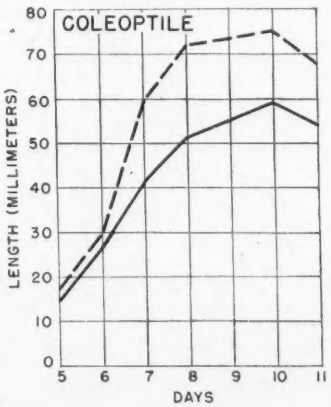

FIGURE 10.—Increase in length of coleoptiles of maize seedlings grown in the dark in two cultures at a constant temperature of 72° F. Solid line, distilled water; broken line, Eaton's solution at double concentration.

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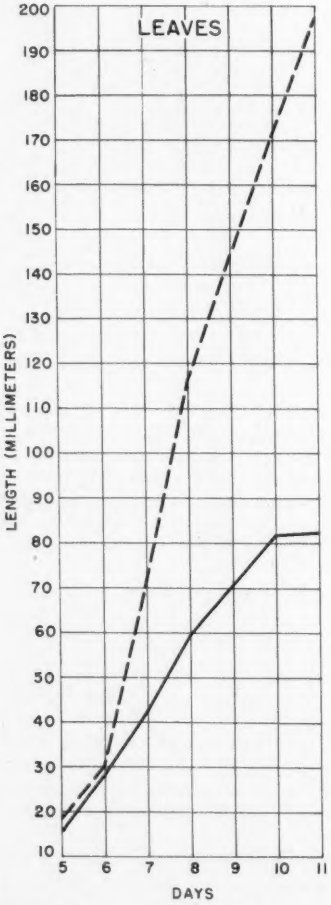

FIGURE 11.—Increase in length of leaves of maize seedlings grown in the dark in two cultures at a constant temperature of 72° F. Solid line, distilled water; broken line, Eaton's solution at double concentration.

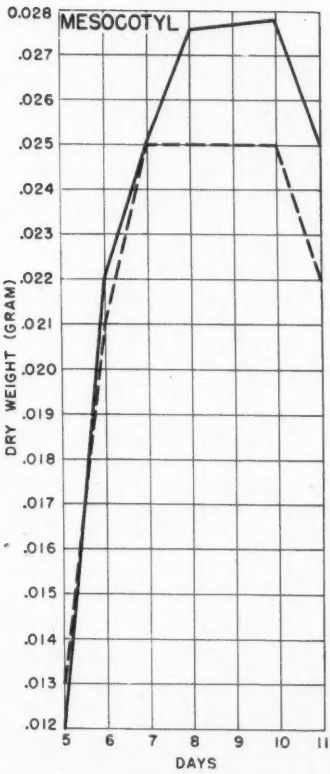

FIGURE 12.—Increase in weight of mesocotyls of maize seedlings grown in the dark in two cultures at a constant temperature of 72° F. Solid line, distilled water; broken line, Eaton's solution at double concentration.

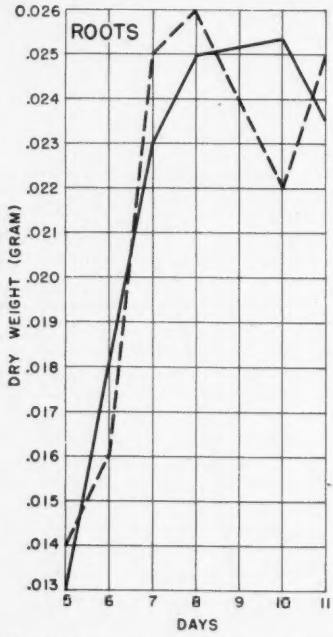

FIGURE 13.—Increase in weight of roots of maize seedlings grown in the dark in two cultures at a constant temperature of 72° F. Solid line, distilled water; broken line, Eaton's solution at double concentration.

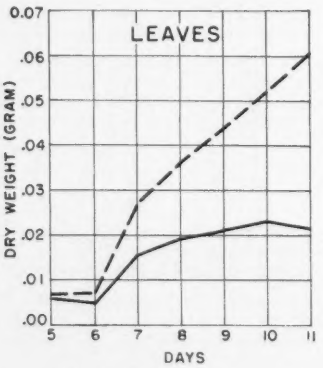

FIGURE 14.—Increase in weight of leaves of maize seedlings grown in the dark in two cultures at a constant temperature of 72° F. Solid line, distilled water; broken line, Eaton's solution at double concentration.

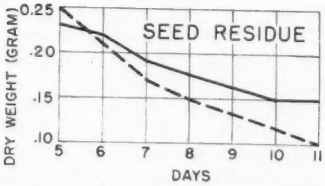

FIGURE 15.—Decrease in weight of seed residue of maize seedlings grown in the dark in two cultures at a constant temperature of 72° F. Solid line, distilled water; broken line, Eaton's solution at double concentration.

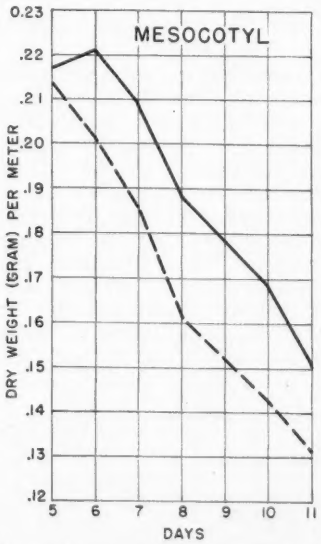

FIGURE 16.—Decrease in weight per meter of mesocotyl of maize seedlings grown in the dark in two cultures at a constant temperature of 72° F. Solid line, distilled water; broken line, Eaton's solution at double concentration.

EFFECT OF DISTILLED WATER AND TWO SALT SOLUTIONS AT TWO CONSTANT TEMPERATURES

In view of the conclusions drawn from the preceding experiment, it was desirable to repeat the test on a larger scale. Three cultures were used, namely, distilled water, the complete solution given on page 184 at double concentration, and the calcium solution given on page 185 at double the concentration. The two chambers used in the experiment described on page 186 were used, but the temperatures were 69° and 90° F. Thermograph records kept during the experiment show these temperatures to have been maintained with a high degree of uniformity.

The seedlings were grown in tin soil cans, 10 weighed seeds of Funk Yellow Dent corn in each can. The cans contained 550 gm. of oven-dry crushed coarse quartz moistened with 110 cc. of solution. The seeds were random samples from a large mixed lot weighed in 10 seed groups and assigned at random to the cans. Analysis of variance showed that the random arrangement had been achieved. Thirty cans of each solution were planted at each of the two temperatures. Within each temperature the cans were randomly arranged. The seeds were planted January 8, 1941, and 5 days after planting 5 cans were sampled from each solution at each temperature every 24 hours for 6 days thereafter. The seedling parts of the individual seedlings were measured and washed free of quartz particles, after which the products of each can were dried at 100° C. and weighed. The weights were not made on an individual-plant basis but on the product of an entire can of 10 seedlings.

The results are shown in table 8, and the apportioned variances are given in table 9. The measurements show that 90° F. is too high a temperature for the sampling system followed. For comparable, useful thermal units, the growth thermometer might be thought of as covering that section of the Fahrenheit scale lying between 50° and 120°. For equal seedling sizes at temperatures of 69° and 90°, sampling of the 90° cultures should be at intervals one-half the length of those at 69°; for example, where the 69° culture was sampled 5 days after planting, sampling of the 90° culture should have started at 2½ days.

TABLE 8.—*Mean length, weight, and percentage of seed and seedling parts of plants grown in the dark for various periods, at different temperatures, and in 3 culture solutions* [1]

Seedling part	5 days from planting						6 days from planting						7 days from planting					
	90° F.			69° F.			90° F.			69° F.			90° F.			69° F.		
	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca sol.
Length mesocotyl...mm..	89.04	147.22	156.67	50.16	64.61	63.37	82.50	149.72	159.26	79.88	107.48	116.38	107.00	156.34	167.32	91.89	142.98	152.37
Length coleoptile...mm..	43.27	67.58	56.02	15.63	16.53	13.90	47.74	64.76	63.44	26.31	34.60	26.60	53.68	66.48	65.06	33.87	47.00	37.35
Length tops...mm..	43.27	101.40	65.98	15.63	16.53	13.90	53.04	138.54	95.30	24.06	34.60	26.60	85.44	198.98	117.66	29.69	51.28	36.92
Weight mesocotyl...gm..	.0181	.0248	.0290	.0125	.0141	.0144	.0180	.0227	.0285	.0200	.0227	.0246	.0183	.0194	.0278	.0224	.0281	.0309
Weight tops...gm..	.0161	.0402	.0273	.0058	.0069	.0064	.0183	.0494	.0357	.0094	.0137	.0105	.0228	.0626	.0379	.0115	.0194	.0140
Weight roots...gm..	.0284	.0271	.0223	.0137	.0128	.0113	.0288	.0313	.0264	.0183	.0171	.0154	.0253	.0275	.0256	.0210	.0202	.0174
Weight per meter of mesocotyl gm..	.2034	.1682	.1850	.2492	.2210	.2268	.2204	.1519	.1790	.2520	.2108	.2114	.1714	.1249	.1680	.2441	.1962	.2072
Weight seed planted...gm..	.3063	.2962	.3080	.3030	.3005	.3014	.3023	.3009	.3022	.3054	.3009	.3002	.3048	.2968	.2985	.3069	.2919	.3004
Weight seed residue...gm..	.1511	.1148	.1426	.2407	.2446	.2421	.1322	.0966	.1164	.2136	.2092	.2154	.1144	.0665	.0870	.1903	.1727	.1869
Weight seed lost...gm..	.0926	.0894	.0869	.0303	.0221	.0272	.1049	.1010	.0952	.0441	.0383	.0342	.1240	.1208	.1202	.0615	.0515	.0512
Weight seed translocated..gm..	.0626	.0920	.0785	.0320	.0338	.0321	.0652	.1033	.0906	.0477	.0534	.0506	.0664	.1095	.0913	.0551	.0677	.0623
Weight dry matter recovered gm..	.2137	.2068	.2311	.2727	.2784	.2742	.1974	.1999	.2070	.2613	.2626	.2660	.1808	.1760	.1783	.2454	.2404	.2492
Recovered dry matter translocated...pct..	29.29	37.96	33.97	11.73	12.14	11.71	33.03	51.68	43.77	18.25	20.34	19.02	36.73	62.22	51.21	22.45	39.20	25.00
Recovered dry matter in mesocotyl...pct..	8.47	11.99	12.55	4.58	5.06	5.25	9.12	11.35	13.77	7.65	9.37	9.25	10.12	11.02	15.59	9.13	11.69	12.40
Recovered dry matter in tops pct..	7.53	19.44	11.82	2.13	2.48	2.33	9.27	24.71	17.25	3.60	5.22	3.95	12.61	35.57	21.26	4.69	8.07	5.62
Recovered dry matter in roots pct..	13.29	13.10	9.65	5.02	4.60	4.12	14.59	15.66	12.75	7.00	6.51	5.79	13.99	15.62	14.36	8.56	8.40	6.98
Dry matter lost...pct..	30.23	30.18	28.21	10.00	7.35	9.02	34.70	33.57	31.50	14.44	12.73	11.39	40.68	40.70	40.27	20.04	17.64	17.04
Dry matter in seed residue pct..	49.33	38.76	46.30	79.44	81.40	80.33	43.73	32.10	38.52	66.44	69.52	71.75	37.53	22.41	29.15	62.01	59.16	62.22
Dry matter translocated..pct..	20.43	31.06	25.49	10.56	11.25	10.65	21.56	34.33	29.98	15.62	17.75	16.86	21.78	36.89	30.59	17.95	23.19	20.74

See footnote at end of table.

TABLE 8.—*Mean length, weight, and percentage of seed and seedling parts of plants grown in the dark for various periods, at different temperatures, and in 3 culture solutions* [1]—Continued

Seedling part	8 days from planting						9 days from planting						10 days from planting					
	90° F.			69° F.			90° F.			69° F.			90° F.			69° F.		
	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca. sol.	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca sol.	Dist. H_2O	E. sol.	Ca sol.
Length mesocotyl...mm...	82.78	158.33	162.08	101.48	151.60	175.94	107.59	169.76	163.47	110.22	158.18	184.56	104.43	164.31	174.65	110.84	164.80	181.72
Length coleoptile...mm...	45.40	65.71	64.20	41.36	63.60	46.64	50.04	64.38	62.63	50.00	66.16	55.60	51.27	67.08	64.59	50.04	70.54	58.18
Length tops.....mm...	74.66	230.29	147.65	44.80	83.96	49.52	83.98	260.94	159.39	59.84	130.43	66.48	146.88	281.71	211.86	77.61	188.80	94.54
Weight mesocotyl...gm...	.0163	.0182	.0245	.0255	.0294	.0351	.0159	.0183	.0225	.0243	.0274	.0334	.0158	.0159	.0213	.0251	.0249	.0323
Weight tops.....gm...	.0202	.0610	.0451	.0165	.0300	.0179	.0180	.0624	.0389	.0180	.0414	.0215	.0270	.0570	.0407	.0222	.0585	.0260
Weight roots.....gm...	.0319	.0252	.0267	.0234	.0229	.0182	.0246	.0245	.0234	.0232	.0244	.0201	.0258	.0238	.0222	.0283	.0309	.0231
Weight per meter of mesocotyl.....gm...	.1989	.1178	.1512	.2521	.1968	.1999	.1473	.1085	.1386	.2277	.1739	.1821	.1532	0974	.1219	.2279	.1517	.1784
Weight seed planted gm...	.2927	.2986	.3096	.3067	.2961	.2961	.3035	.2921	.3088	.3067	.3030	.2976	.3120	.3064	.3122	.3033	.3032	.3054
Weight seed residue.gm...	.0870	.0561	.0745	.1785	.1462	.1704	.0887	.0512	.0729	.1621	.1246	.1535	.0706	.0465	.0627	.1396	.0898	.1342
Weight seed lost....gm...	.1372	.1378	.1388	.0629	.0676	.0545	.1563	.1357	.1511	.0790	.0852	.0692	.1728	.1631	.1654	.0881	.0991	.0897
Weight seed translocated gm...	.0685	.1047	.0963	.0653	.0823	.0712	.0585	.1052	.0848	.0656	.0932	.0749	.0686	.0968	.0841	.0756	.1143	.0815
Weight dry matter recovered.....gm...	.1555	.1608	.1708	.2438	.2285	.2416	.1472	.1564	.1577	.2277	.2178	.2283	.1392	.1433	.1468	.2152	.2041	.2157
Recovered dry matter translocated.....pct...	44.05	65.11	56.38	26.78	36.02	29.47	39.74	67.26	53.77	28.81	42.79	32.81	49.28	67.55	57.29	35.13	56.00	37.78
Recovered dry matter in mesocotyl.....pct...	10.48	11.32	14.34	10.54	12.87	14.53	10.80	11.70	14.27	10.67	12.58	14.63	11.35	11.10	14.51	11.66	12.20	14.97
Recovered dry matter in tops.....pct...	12.99	37.94	26.41	6.77	13.13	7.41	12.23	39.90	24.67	7.91	19.01	9.42	19.40	39.78	27.72	10.32	28.66	12.05
Recovered dry matter in roots.....pct...	20.51	15.67	15.63	9.60	10.02	7.53	16.71	15.66	14.84	10.19	11.20	8.80	18.53	16.61	15.12	13.15	15.14	10.71
Dry matter lost.....pct...	46.87	46.15	44.83	20.51	22.83	18.41	51.50	46.46	48.93	25.76	28.12	23.25	55.38	53.23	52.98	29.05	32.68	2.937
Dry matter in seed residue.....pct...	29.72	18.79	24.06	58.20	49.38	57.55	29.23	17.53	23.61	52.85	41.12	51.57	22.63	15.18	20.08	46.03	29.62	43.94
Dry matter translocated pct...	23.40	35.06	31.10	21.29	27.79	24.05	19.28	36.02	27.46	21.39	30.76	25.17	21.99	31.59	26.94	24.93	37.70	26.69

[1] Dist. H_2O = distilled water; E. sol. = Eaton's solution (p. 184) doubled in concentration; Ca sol. = solution of calcium salts (p. 185) doubled in concentration.

TABLE 9.—*Analyses of variance based on means of 10 seedling groups for the seedling parts and the treatments shown*

Source of variation	Degrees of freedom	Mean square									
		Length			Weight						
		Mesocotyl	Coleoptile	Leaves	Mesocotyl	Leaves	Roots	Per meter of mesocotyl	Seed residue	Dry matter lost	Dry matter translocated
Total	179	1, 593. 81	272. 48	5, 313. 54	0. 000037	0. 00031	0. 000029	0. 00193	0. 00628	0. 001963	0. 00062
Culture	2	[1] 65, 325. 48	[1] 3, 586. 15	102, 541. 59	[1] .000910	[1] .00933	[1] .000205	[1] .04236	[2] .01292	[1] .000566	[1] .01103
Temperature	1	[1] 12, 036. 05	[1] 13, 267. 94	291, 998. 77	[1] .000716	[1] .01523	[1] .001647	[1] .13890	[1] .33547	[1] .213880	[1] .01883
Time	5	[1] 13, 700. 85	[1] 2, 624. 41	[1] 6, 077. 71	[1] .000142	[1] .00179	[1] .000145	[1] .01388	[1] .04182	[1] .021292	[1] .00386
Interactions:											
Culture-temperature	2	[1] 1, 677. 01	[1] 429. 57	[1] 23, 450. 53	[1] .000014	[1] .00171	.000010	[1] .00164	.00058	[1] .000294	[1] .00175
Culture-time	10	[1] 435. 95	40. 17	4. 055. 86	[2] .000010	[1] .00021	[2] .000010	[1] .00064	.00033	.000006	.00019
Temperature-time	5	[1] 11, 791. 93	[1] 1, 928. 42	2. 013. 23	[1] .000496	[1] .00043	[1] .000298	[2] .00065	.00247	[1] .000280	[1] .00287
Solution-temperature-time	10	[1] 535. 17	[1] 77. 68	1, 058. 96	[1] .000019	[1] .00015	[1] .000017	.00034	.00055	[1] .001503	.00017
Error	144	176. 11	24. 53	292. 16	.000004	.00002	.000004	.00025	.00369	.000009	.00021

[1] $P<0.01$.
[2] $P<0.05$.

The results of this experiment did not confirm the data, obtained from the preceding experiment, on the effect of the complete solution on length of mesocotyl. A comparison of figures 9 and 17 shows that, while the growth curves in the two experiments are very similar in rate and final size for the plants grown in the complete culture solution, they are astonishingly different for the plants grown in the distilled water. In the experiment illustrated in figure 9, the length of mesocotyl in distilled water is equal to that in the complete solution and both are very long.

For the seedling parts above ground, the analyses of variance (table 9) show significant interactions of solutions with temperature. The nature of these interactions is shown in figures 17 to 21. With the exception of measures of mesocotyl development, at 69° F. the plants in the calcium solution were more nearly like those in distilled water, and at 90° these plants were intermediate between those in distilled water and those in the complete solution. The root development shows no interaction of solution with temperature, though there is a significant triple interaction of solution, temperature, and time.

Length of Mesocotyl

It is evident that at the first sampling date the mesocotyls in all three solutions in the 90° F. culture were approaching their maximum length. Only the upper reaches of the curves are available (fig. 17, *A*). It is clear, however, that the solution of calcium salts produces the longest mesocotyls, although there is not much difference in this respect between the two nutrient solutions. In the 69° culture both nutrient solutions clearly accelerated the elongation of the mesocotyls and carried them to a greater final length than did distilled water (fig. 17, *B*). This is not in accordance with the results first obtained (p. 197 and fig. 9). The calcium solution was more effective in stimulating the elongation of the mesocotyl than was the complete solution, thus confirming the results of the previous experiments.

Length of Coleoptile

Both solutions were more effective than distilled water in producing long coleoptiles at 90° F. The complete solution produced the longest coleoptiles, but the two solutions were very similar in this respect (fig. 18, *A*). At 69° the differences in coleoptile length between the three solutions were not so great (fig. 18, *B*). The coleoptiles in the calcium solution and in distilled water were much more nearly the same size at 69° than at 90°. As at 90°, the complete solution produced the longest coleoptiles at 69°.

Length of Tops

At 90° F. the true leaves were very much longer in the complete solution and grew faster than in the other solutions (fig. 19, *A*). The calcium solution produced leaves midway in length between those in the complete solution and those in distilled water. At 69° the lengths of leaves in the calcium solution and in the distilled water were very nearly alike, whereas the complete solution produced long, rapidly elongating leaves (fig. 19, *B*).

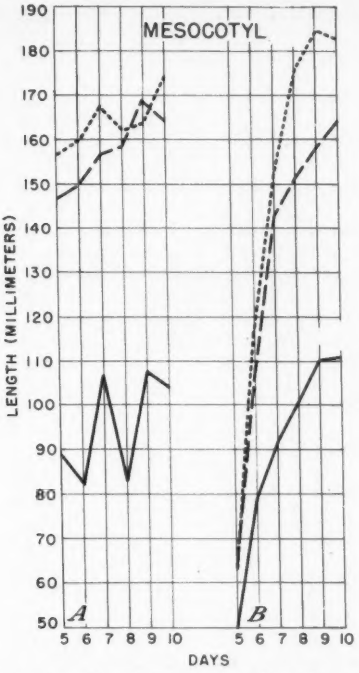

FIGURE 17.—Increase in length of mesocotyls of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

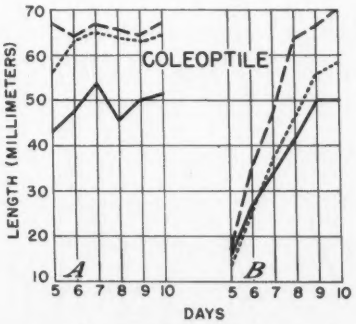

FIGURE 18.—Increase in length of coleoptiles of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

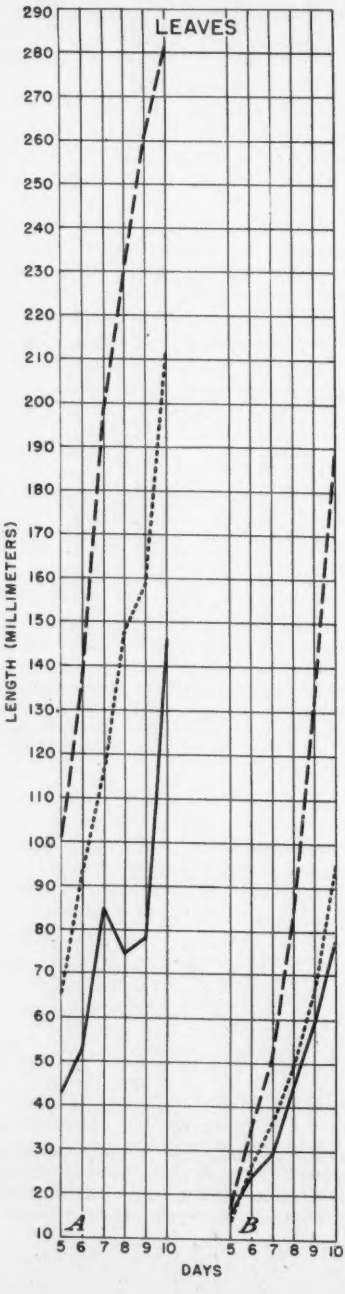

FIGURE 19.—Increase in length of leaves of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

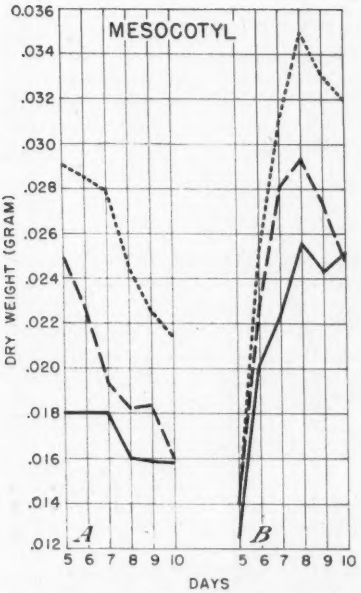

FIGURE 20.—Increase in weight of mesocotyls of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

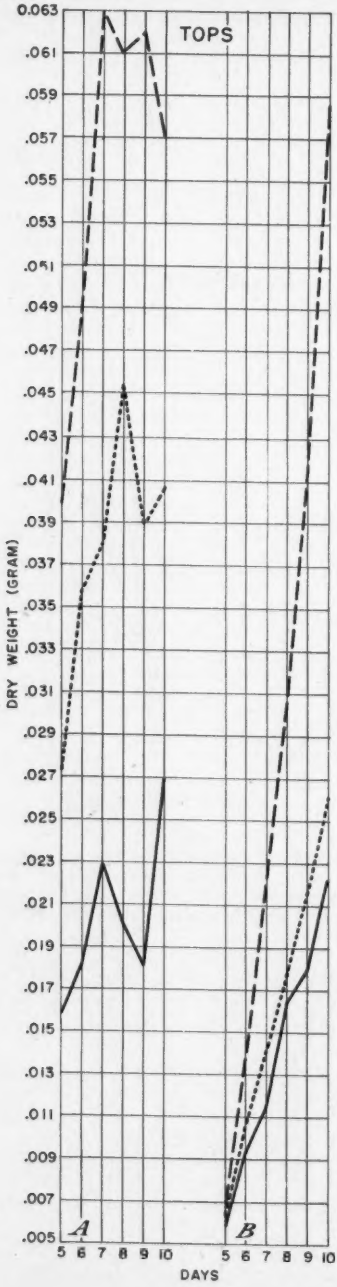

FIGURE 21.—Increase in weight of tops of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

Weight of Mesocotyl

The relatively late sampling period for the 90° F. culture is shown graphically in figure 20, *A*, where the dry weights of mesocotyls are greatest on the first sampling day and decline each day thereafter. The mesocotyls were heaviest in the calcium solution, which would be expected to follow from the greater length of this organ in this solution. The loss in dry matter is probably the result of the drain made by the rapidly elongating leaves, which doubtless pull sustenance from the mesocotyl as well as from the seed. In the 69° culture the dry weights of mesocotyls increase with time in all three solutions, until at 9 days after planting all three start to decline (fig. 20, *B*). The leaves at this temperature are exserted from the coleoptile 7 days after planting, but apparently the seed is able to provide food for both leaves and mesocotyls as long as these organs are located close to the source of supply. At 8 days the leaf node is 16 to 18 cm. above the seed in the culture solutions and the mesocotyl begins to lose dry matter to the leaves. At 69°, as at 90°, the dry matter in the mesocotyls is greatest in the calcium solution.

Weight of Tops

The weight of the tops at 90° F. reached its maximum in the complete solution 7 days after planting and in the calcium solution 8 days after planting (fig. 21, *A*). In distilled water the weights were irregular, but the heaviest plants were obtained on the last sampling date, 10 days after planting. The weight of dry matter represented by the leaves was greatest in the complete solution, least in distilled water, and almost the mean of these two in the calcium solution. At 69° the greatest weights in all three solutions were obtained 10 days after planting (fig. 21, *B*). Here distilled water and the calcium solution were not greatly different in the dry weight of leaves produced. The complete solution was outstanding, with leaves twice as heavy as those in the calcium solution.

Weight of Roots

The weight of roots in the 90° F. culture was very irregular, but the heaviest roots were found in distilled water and the lightest in the calcium solution (fig. 22, *A*). The weight of roots in complete solution was very similar to that in the distilled water. The weight declined in all three cultures before the final sampling date, indicating a loss of dry matter to the upper parts of the seedling. In the 69° culture the weight of roots increased rapidly with time in all three solutions and was greatest at the close of the experiment (fig. 22, *B*). The calcium solution was distinctly a poor medium for root development in comparison with the complete solution and the distilled water, which were very similar in their effect on roots.

Weight of Mesocotyl per Unit Length

As a means of expressing the weight per unit of length of mesocotyls, the weight per meter was chosen. At both temperatures and in all solutions, the weight per meter declined with time (fig. 23, *A* and *B*). As the mesocotyls increased in length, they decreased in dry weight per meter. The weight per meter was greatest in the distilled water

at both temperatures and for all sampling periods and conversely was lightest in Eaton's solution.

From one point of view it could be assumed that a certain amount of dry matter was apportioned to the mesocotyl, and, as this organ became longer with age or by nutritional stimulation, the amount of dry matter per unit length necessarily became less. However, the calcium salts produced the longest mesocotyls but not the lightest in weight per unit of length, and the actual weight of dry matter in the mesocotyl was greater in the two solutions than in distilled water. The most probable explanation for the loss in unit weight with time and with nutritional stimulation is that both these conditions advance

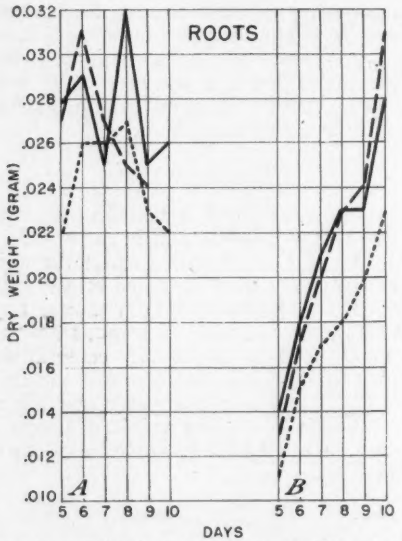

FIGURE 22.—Increase in weight of roots of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

the seedling ontogeny, with the result that the developing leaves begin to constitute a drain on the nutrients in the mesocotyls. Under these conditions weight per unit length would decrease with length and time.

WEIGHT OF SEED RESIDUE

The amount of material remaining in the seed, of course, decreased with time. At 90° F. the reserve material remaining in the seed was greatest in distilled water and least in Eaton's solution (fig. 24, *A*). The calcium solution was approximately midway between these two at all sampling periods. The curves show that for this temperature the maximum rate of decline had been reached at the time of the first sampling date, and at 7 days after planting the rate of decline had distinctly slowed.

At 69° F. the solutions stood in the same order as at 90°, but the distilled water and calcium solution were almost identical (fig. 24, *B*). The rate of decline was much greater in all solutions at this temperature than at 90°, but this doubtless is due to the relatively earlier

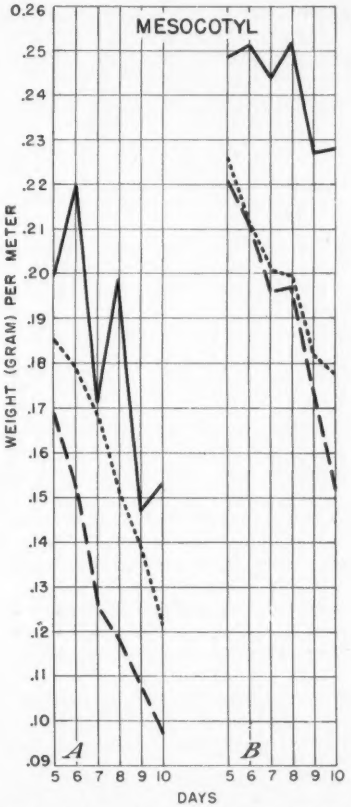

FIGURE 23.—Decrease in weight per meter of mesocotyl of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

sampling period. At the close of the experiment there was no evidence of a slackening in rate of decline.

WEIGHT OF DRY MATTER RECOVERED AND OF DRY MATTER LOST

The weights of dry matter recovered and of dry matter lost are complementary. The amount of dry matter recovered declines rapidly with time, and, conversely, the amount of dry matter lost

increases rapidly with time (fig. 25, *A*, *B*, and fig. 26, *A*, *B*). In measurements of this nature there are necessarily some unavoidable losses due to handling, despite meticulous care. A certain loss of small hair roots is known to have occurred, and the total weight lost

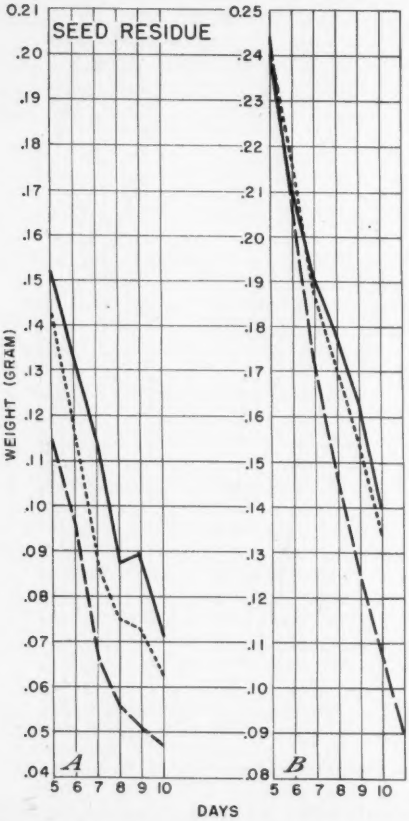

FIGURE 24.—Decrease in weight of seed residue of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

through this factor would increase with the increasing size of the seedlings, because as the plants became larger not only were there more roots to lose but also it was more difficult to separate the seedlings from the quartz. More agitation in water was required to remove the quartz, and consequently there was an increased loss of small roots.

However, only a fraction of the lost dry matter can be accounted for by losses in handling. The principal cause of loss was undoubtedly the presence of micro-organisms; this was reflected in the greater losses at the higher temperatures, as the cultures were not sterile.

A second large factor in the loss of weight must be the energy utilized in the process of translocation with elongation, starch con-

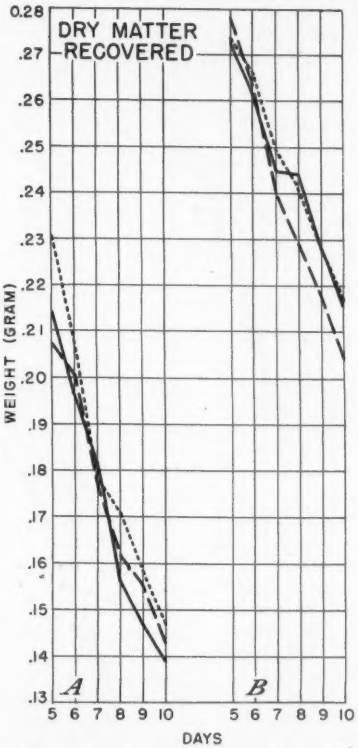

FIGURE 25.—Decrease in weight of total dry matter recovered from maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

version, oxidation, etc. There is a third factor, namely, the loss of solutes to the solutions. Were this an important factor, the distilled-water cultures would show the greatest loss, and to some extent they do, although the differences between the three cultures are not consistent at all sampling dates.

WEIGHT OF DRY MATTER TRANSLOCATED

In both temperature series the greatest quantity of dry matter removed from the seed to the plant occurred in Eaton's solution (fig.

27, *A*). At 90° F. in the complete solution the maximum amount of dry matter had been translocated 7 days after planting, whereas in the calcium solution and in the distilled water the maximum was reached at 8 days. The calcium solution stood almost midway between the distilled water and the complete solution at 90° but not at 69°, where the curves for distilled water and calcium are closely parallel. At 69° in the complete solution the rate of dry matter trans-

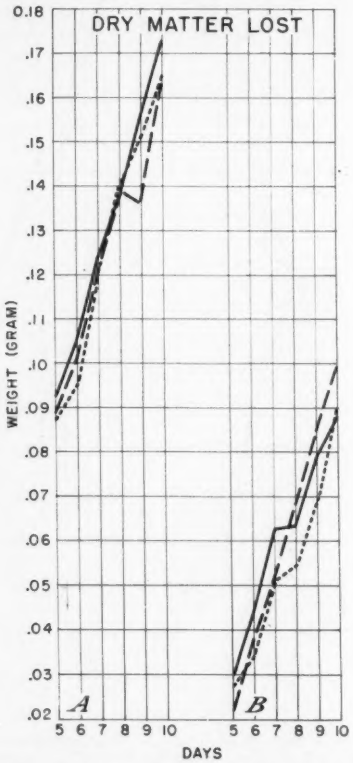

FIGURE 26.—Increase in weight of total dry matter lost of maize seedlings grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

located was increasing at the close of the experiment, and the amount of translocated material actually exceeded that for the same solution at 90° (fig. 27, *B*).

PERCENTAGE OF RECOVERED DRY MATTER TRANSLOCATED TO THE SEEDLING

Of the dry matter recovered, the largest percentage translocated to the seedling is found in Eaton's solution at 90° F., where almost 70 percent of the dry matter left at the close of the experiment was found in the seedling (fig. 28, *A*). At this temperature the calcium solution

stood midway between the distilled water and the complete solution. All three groups had passed their maximum rates by the seventh or eighth day from planting.

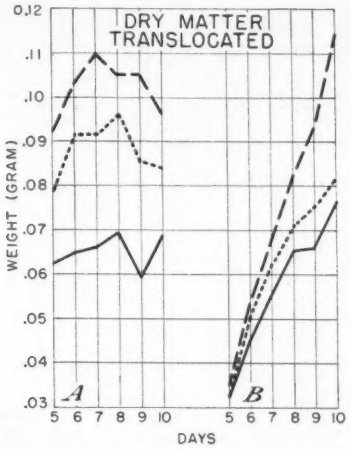

FIGURE 27.—Increase in weight of total dry matter translocated from seed to seedling of maize grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

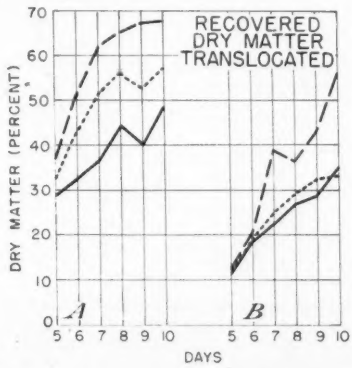

FIGURE 28.—Percentage of recovered dry matter translocated from seed to seedling of maize grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

At 69° F. the solutions stood in the same order. Even in the complete solution less than 60 percent of the recovered dry matter was found in the seedlings, although the rate was still rising for this solution (fig. 28, *B*). For the distilled water and the calcium solution, which were very closely alike, the rates of transfer had apparently reached

a maximum at the close of the experiment at a level well below that reached at the higher temperature.

PERCENTAGE OF TOTAL RECOVERED DRY MATTER IN TOPS

At the close of the experiment 40 percent of the recovered dry matter was found in the leaves and stems of the seedlings growing in the com-

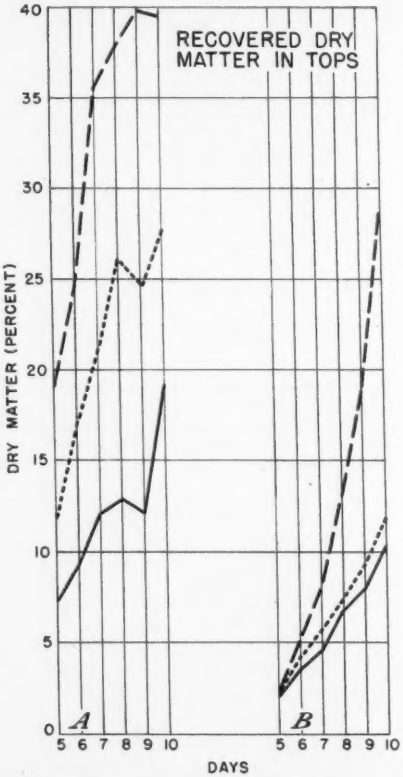

FIGURE 29.—Percentage of recovered dry matter found in tops of seedlings of maize grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

plete solution at 90° F., and evidently the maximum point had been reached (fig. 29, *A*). The curve for the seedlings growing in the calcium solution falls almost midway between that for distilled water and that for the complete solution.

At 69° F. none of the cultures had reached the maximum point at the close of the experiment. Here the curves for the calcium solution and for the distilled water lie close together and are very similar (fig. 29, *B*).

Percentage of Total Recovered Dry Matter in Roots

There was very little difference between the cultures in the percentage of dry matter translocated to the roots, except that at 90° F. the roots in the distilled water clearly exceeded those in the other cultures (fig. 30, *A*). At 69° the percentage of dry matter found in the roots was greatest in the complete solution, though not outstandingly so (fig. 30, *B*). The percentages were uniformly higher at 90°, though at this temperature the maximum point evidently had been

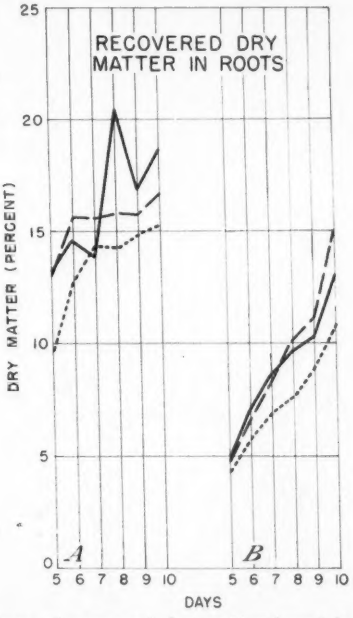

Figure 30.—Percentage of recovered dry matter found in roots of seedlings of maize grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

reached for all three cultures at the time the experiment was terminated. This point had not been reached for any of the cultures at 69°.

Percentage of Total Recovered Dry Matter in Mesocotyl

At 90° F. the percentage of dry matter in the mesocotyl was greatest for the calcium solution and least for the distilled water (fig. 31, *A*). In general, the percentage declined in the complete solution from the first samples, though the decline was irregular. The lack of agreement in the form of the three curves at 90° may have resulted from the rapid expansion of the leaves in the complete solution in comparison with those in the other two.

At 69° F. the three solutions produced comparable curves until the last two sampling periods, when the percentage declined in the complete solution (fig. 31, *B*).

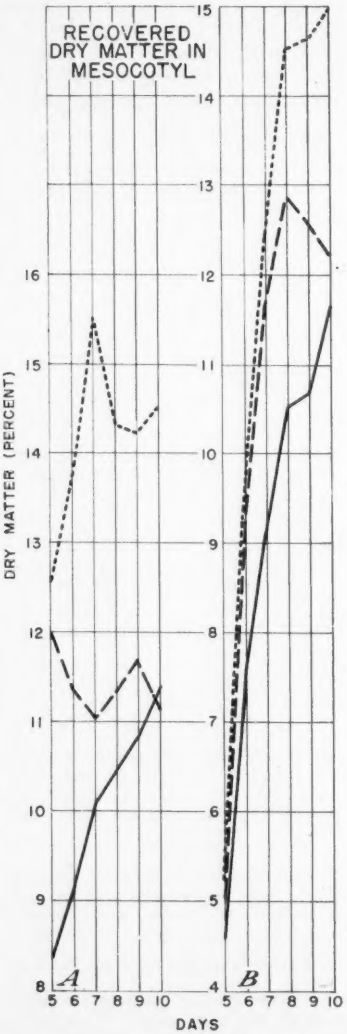

FIGURE 31.—Percentage of recovered dry matter found in mesocotyls of seedlings of maize grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

Percentage of Dry Matter Translocated

The percentage of dry matter translocated from the seeds to seedling parts is shown in figure 32, *A* and *B*. At 90° F. the three solutions stood in the order of Eaton's, calcium, and distilled water, the first-named having the highest percentage. The three curves are very similar in shape, with the maximum percentage at about 8 days after planting. The decline that sets in after this period is the result

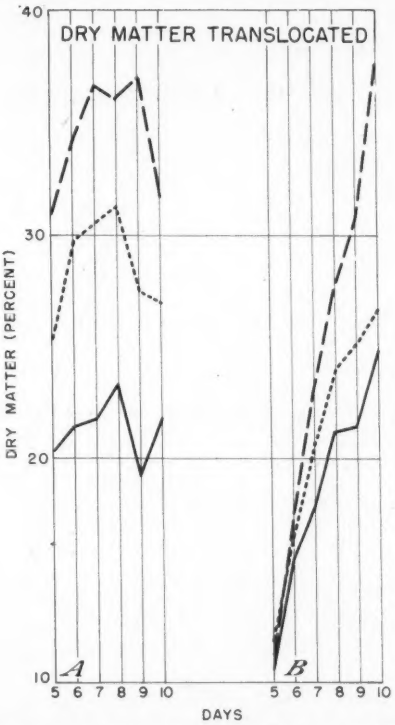

Figure 32.—Percentage of original dry matter translocated from seed to seedling of maize grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

of dry matter lost, probably chiefly to fungi and bacteria. However, this does not furnish a complete explanation, as at 69° the percentage of dry matter translocated increased fairly steadily in all three cultures, and in the complete solution on the last sampling date actually exceeded the maximum found in the 90° group. Further, reference to figure 26, *A* and *B*, shows the two temperature groups to have very similar loss curves. It should be noted that the maximum percentage of dry matter actually moved from the seed to the seedling is less than 40 percent in the most effective culture and less than 25 percent in distilled water.

Percentage of Dry Matter in Seed Residue

Of the dry matter in the seed when planted, that not translocated to the seedling was either left in the seed or lost. Of course, part of the seed material is not usable by the seedling and part of it, though used, remains in the seed, such, for example, as that in the scutellum. At 90° F., at all sampling dates, the seed residues were least in the complete solution and greatest in distilled water, in line with the seedling sizes and percentages of dry matter translocated. It is quite evident from figure 33, *A*, that the decline in weight of seed residue

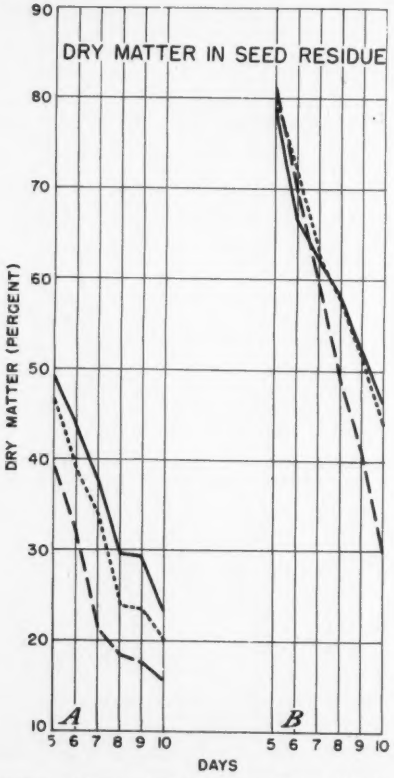

Figure 33.—Percentage of dry matter in seed residue of maize grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

had set in 7 or 8 days after planting.

At 69° F. the percentage of dry matter left in the seed was least in the complete solution. The curves for distilled water and the calcium solution are almost identical (fig. 33, *B*). The rate of loss had not slackened in any of the three cultures, but even in the complete

solution 30 percent of the original dry matter was still to be found in the seed.

Percentage of Dry Matter Lost

The percentage of dry matter lost has been discussed on pages 215–217. The graphs (fig. 34, *A* and *B*) serve to strengthen the conclusion

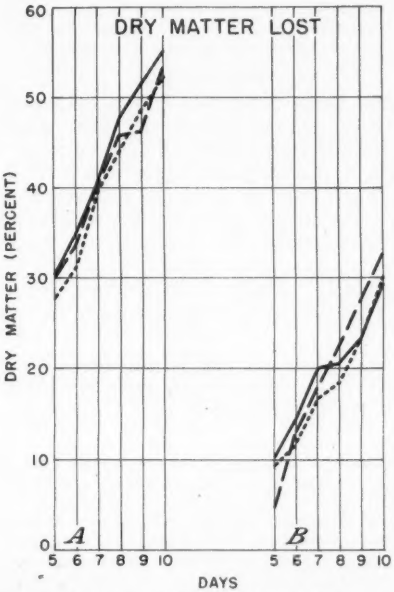

Figure 34.—Percentage of original dry weight of feeds not recovered in seed residue and seedlings of maize grown in the dark in three cultures (p. 204). Solid line, distilled water; broken line, Eaton's solution at double concentration; dotted line, calcium solution at double concentration. Cultures grown at a constant temperature of (*A*) 90° F., (*B*) 69°.

that this factor was not greatly influenced by the nature of the culture solution and to bring out the fact that the percentage of dry matter lost was very much higher in all solutions at 90° F. than at 69°. There is some evidence that the greatest percentage of dry matter was lost in distilled water at the higher temperature and in the complete solution at the lower temperature.

Germination

Neither the cultures nor the temperatures had any effect on germination percentage, which was 99 for the entire experiment. Although germination was lowest in distilled water and highest in the complete solution, the difference between these two solutions was not significant ($\chi^2=0.04$). The distribution of germinated seeds is given in table 10.

TABLE 10.—*Distribution of germinated seeds in the solutions and temperatures shown*

Temperature (° F.)	Solution			Total
	Distilled water	Eaton's	Calcium	
69	294	299	298	891
90	296	298	295	889
Total	590	597	593	1,780

DISCUSSION

It is evident from these experiments that maize seedlings grown in the dark are benefited by certain nutrient salts, and further that the kind of salts determines the part of the seedling to be benefited. The action of the salts is reflected not only in the total amount of dry matter moved into different parts of the seedling but also in the rate of its movement. Seedlings grown in distilled water, for example, have mesocotyls heavier per unit of length than those grown in the several nutrient solutions, a condition which indicates a retarded movement of dry matter along the axis. In distilled water also the leaves appear later and do not reach, in a given time, the size of those in the salt solutions.

Bonner (*1*) measured the growth, by means of curvature, of excised coleoptiles of *Avena* when immersed in various solutions. He found a more rapid growth in an acid medium (pH 4.1) and reached the following conclusions:

> * * * It would appear then that there are at least two ways connected with growth substance in which the growth rate of an *Avena* coleoptile can be increased. The first is the way in which it is done in the normal plant, by an increase [in] the total amount of growth substance which is present. Only a small portion of this will be in the active non-dissociated form at the pH of the plant, but by increasing the total amount present, the amount present as non-dissociated acid will also be increased. The second way in which the growth rate may be increased is by conversion of some of the inactive growth substance salt already present in the plant into the acid. This is what occurs in the "acid growth" reaction, when the cells of the plant are actually made more acid, and the ionization of the growth substance reduced.

Thimann and Schneider (*7*), using peeled excised coleoptiles of *Avena* and stems of *Pisum* floating in the solution, demonstrated an increased growth for various concentrations of potassium chloride.

No attempt was made in any of the experiments herein reported to maintain the salt concentrations present at the start. The seedlings were enclosed in tin cylinders, which were unopened from the time of planting until the plants were measured. In consequence, growth in the salt solutions may have been checked either by the exhaustion of the required salts or by an excessive concentration through water loss. Although the tin cylinders retarded the loss of water from the system, the actual quantity of condensed water on the inside of the containers and on the plants themselves constituted a loss sufficient to bring about a real increase in the concentration of the culture solutions.

Steward and Preston (*6*) studied the effect of salt concentration on the metabolism of potato disks and reached the following conclusions:

> 2. The effects of salt concentration on starch hydrolysis are slight. The tendency is for increased concentration of potassium salts to decrease and calcium salts to increase starch hydrolysis. This is the converse of the more important effect of salts on all the processes which involve oxidation.

3. Increased external concentration of potassium salts increases respiration and all other reactions which are favored by oxygen, whereas corresponding concentrations of the calcium salts with a common anion depress these processes.
4. The salt concentrations which induce high respiration do not produce high sugar content. High rates of respiration and lower sugar content obtain in the tissue exposed to strong potassium salts and the converse is true of the disks treated with calcium salts. Sugar concentration does not determine respiration.
5. The effective ions of the salts are the cations. The specific effects of the cations are accentuated by the anions and these, like the contrast between the effect of potassium and calcium salts at the same equivalent concentration, are influenced by the anions in the order $NO_3 > Cl > Br > SO_4$ which is also the order in which they influence absorption of a common cation.
6. The effects of salts on respiration are closely connected with their effect on protein synthesis from stored amino acids. Potassium salts stimulate, calcium salts depress both processes.

The behavior of these potato disks may be closely analogous to the processes that take place in maize seedlings growing in the dark. Chemical analyses were not made of the seedling parts, but Frey-Wyssling and Blank (*4*) have shown, with the coleoptile of maize, that water-soluble and protein nitrogen migrate from the seed into the coleoptile as long as it is growing.

In potato disks protein synthesis is depressed in calcium solution relative to potassium salts, and if this process holds true for maize it might explain the retarded growth of coleoptiles and leaves in the calcium solutions tested. However, it should be noted that there is an interaction of solution with temperature of such a nature that the calcium salts at 69° F. have relatively less effect on leaf size than at 90°.

The development of roots, as measured by their dry weight, is unaffected by Eaton's solution (tables 6–8 and figs. 13 and 22). However, the solution of calcium salts is obviously detrimental. White (*8*) showed, with excised root-tip cuttings of wheat growing in Upsenski solution, that growth could be improved by lowering the calcium by as much as 50 percent.

Naylor (*5*) studied the effects of temperature, calcium, and arsenous acid on seedlings of *Poa pratensis* growing in the light and reached the general conclusion that calcium in large quantities is detrimental to young seedlings. Once the seedlings are established, however, calcium stimulates rapid growth. The detrimental effect, at least in the early seedling stages, may be the result of root inhibition. Certainly, with maize, in those cases where seedling survival depends upon a maximum extension of the mesocotyl, calcium is beneficial.

Day (*2*) showed, with *Pisum sativum*, that plants starved for calcium are dwarfed, and apparently this element plays an important role in the stem elongation.

The morphology of the maize seedling is such that the mesocotyl has an upper limit of length, as does also the coleoptile. Under ordinary conditions, neither of these organs reaches the maximum length. To a certain degree they elongate together, though in the early stages the rate of elongation is more rapid in the mesocotyl. The elongation of the coleoptile continues after that of the mesocotyl stops. By the time the first leaf is exserted from the coleoptile, extension of the mesocotyl is complete.

Under all except optimum conditions, the mesocotyl does not reach its full possible length and the leaves begin growth. As the leaves are not exserted from the coleoptile until the mesocotyl has stopped elongating, conditions that speed the growth of this organ speed the appearance of the leaves. Once the leaves have begun to increase in

size rapidly, the mesocotyl shrinks somewhat in length and noticeably in diameter. This shrinkage in size is accompanied by a loss of dry matter.

Where two solutions are used, one of which stimulates the development of the mesocotyl and the other the development of the leaves, it is evident that in the one case leaf growth will be delayed by the developing mesocotyl and in the other case the full length of the mesocotyl will not be reached because of the nutrition requirements of the rapidly expanding leaves.

Although these experiments were terminated before the translocation of dry matter was complete, there is little reason to believe that the leaves in the calcium solution would ever have reached the dry weight of those in the complete solutions. The inhibitory effect of calcium on leaf development, as compared with the more complete solutions, cannot be attributed entirely to the delay in leaf growth brought about through the stimulation of the mesocotyl by this element.

SUMMARY AND CONCLUSIONS

Maize seedlings grown in the dark were benefited by certain salts in the culture medium.

These salts increased the rate of transfer, and the total quantity translocated, of the dry matter stored in the seed.

Calcium salts stimulated the elongation of the mesocotyl but did not produce the longest or heaviest leaves.

Leaf growth was stimulated at the expense of the mesocotyl by all except calcium salts.

Coleoptiles and leaves apparently reacted to the same solutions.

Dry weight of roots was not increased by the salts used and was depressed by calcium salts.

The effects produced by the salts used were dependent on temperature in the range 69° to 90° F. In certain respects calcium salts are relatively less effective in stimulating growth at the lower temperature.

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